bond with the backbone, resulting in a cyclic conformation with a fixed alignment of the two carbonyls. The amidated form of glutamine has been studied at varying pH and temperature; data has been compared to that obtained for amidated alanine, amidated asparagine and non-amidated glutamine.

**1595-Pos Board B439**
**Chemical Exchange 2DIR of Base Pair Opening Fluctuations in RNA Tetraloops: A Simulation Study**
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Hydrogen bonds play an important role in RNA structure and dynamics. Fluctuations in base pair openings could be the starting point of unfolding processes or could indicate potential docking sites for ligands or proteins. The effects of hydrogen bond formation and breaking kinetics in RNA base pairs on the linear and coherent third order infrared spectra of small UUCG tetraloops in solution can be described by Markovian, not necessarily Gaussian, fluctuations. We have simulated these spectra using the stochastic Liouville equations. Slow fluctuations are described phenomenologically. Fast fluctuations are characterized by an N-state jump model for hydrogen bond configurations, where N depends on the specific tetraloop. Bases in the RNA strands that exhibit high levels of fluctuation are isotope labeled and the chemical exchange 2DIR spectra are calculated. The existence and evolution of the resultant cross peaks at different waiting times provides information on the coupling interactions between base pairs in the loops, which will be used to help characterize unfolding mechanisms of the RNA strands.

**1596-Pos Board B440**
**Nitrile-Modified Nucleosides as a Probe of Local Nucleic Acid Environments**
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The nitrile functional group has been extensively investigated as a probe in proteins but not as a probe in nucleosides. The solvent-induced vibrational frequency shift of the nitrile band of 5-cyano-2′-deoxyuridine was examined in THF-$H_2O$ mixtures. The nitrile stretching frequency ($\nu_{C\equiv N}$) exhibited moderate solvent sensitivity, undergoing a 9.2 $cm^{-1}$ blue shift from THF to $H_2O$, and varied linearly with temperature, exhibiting a 1.4 $cm^{-1}$ red shift from 290 K to 340 K in $H_2O$. The $\nu_{C\equiv N}$ of 5-cyano-2′-deoxy-3′,5′-bis-O-(*t*-Bu$Ph_2$Si)-uridine underwent a 1.3 $cm^{-1}$ blue shift when titrated with a base-pairing mimic, 2,6-diheptanamido-pyridine to yield an association constant of 90 $M^{-1}$. The corresponding $C^{15}N$ labeled nucleoside is currently under investigation by $^{15}N$ NMR to determine the utility of the $C^{15}N$ moiety as an NMR probe of the local environment of nucleic acids. Both the IR and NMR results will be presented complemented by density functional theory calculations.

**1597-Pos Board B441**
**Multidimensional Optical Spectroscopy Of Proteins Out Of Thermal Equilibrium**
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In recent years, nonlinear multidimensional optical spectroscopy has been used as a highly sensitive probe of molecular dynamics in the condensed phase. Multidimensional optical spectroscopy builds upon the methodology of two-dimensional nuclear magnetic resonance spectroscopy and applies the same principles to vibrational and electronic resonances such that these techniques may be used as an ultrafast probe of molecular dynamics. In particular, these techniques have been used to study the thermal unfolding of proteins following a nanosecond temperature jump. In this study, we examine the multidimensional optical spectra of several biological systems of interest out of thermal equilibrium by using molecular dynamics to develop snapshots of the systems and the SPECTRON software package to calculate the spectroscopic signals. In order to enhance conformational sampling, an artificial temperature is used; the exact correlation functions of the system contributing to the material response are recovered using an action-reweighting scheme based on a stochastic path-integral formalism. The calculated spectra provide information on the states sampled by the system during the course of thermal unfolding.

## Biotechnology & Bioengineering I

**1598-Pos Board B442**
**Spatially-resolved Analysis Of DNA Nanocomplex Self-assembly Enabled By Integrating Nanophotonics And Microfluidics**
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Advances in genomics continue to fuel the development of future therapeutics that can target pathogenesis at the cellular and molecular level. Often functional only inside the cell, nucleic acid-based therapeutics require an efficient intracellular delivery system. One widely adopted approach is to complex DNA with a gene carrier to form nanocomplexes via electrostatic self-assembly, facilitating cellular uptake of DNA while protecting it against degradation. The challenge, however, lies in rational design of gene carriers, since premature dissociation or overly stable binding would be detrimental to the cellular uptake and therapeutic efficacy. Nanocomplexes synthesized by bulk mixing showed a diverse range of intracellular unpacking and trafficking behavior, which was attributed to the heterogeneity in size and stability of nanocomplexes. The heterogeneity of nanocomplexes resulting from bulk synthesis hinders the accurate assessment of the self-assembly kinetics and adds to the difficulty in correlating their physical properties to transfection efficiencies or bioactivities. We present a novel convergence of nanophotonics (i.e. QD-FRET) and microfluidics to characterize kinetic aspect of the nanocomplexes synthesis under laminar flow in real-time. QD-FRET provides a highly sensitive and quantitative indication of the onset of molecular interactions and throughout the process of nanocomplexes synthesis, whereas microfluidics offers a well-controlled microenvironment to spatially analyze the process with high temporal resolution (~milliseconds). For the model system of polymeric nanocomplexes, two distinct stages in the self-assembly process were captured by this analytic platform. The kinetic aspect of the self-assembly process obtained at the microscale would be particularly valuable for microreactor-based reactions which are relevant to many micro- and nano-scale applications. Further, customized nanocomplexes may be generated through proper design of microfludic devices, and the resulting QD-FRET polymeric DNA nanocomplexes could be readily applied for establishing structure-function relationships.

**1599-Pos Board B443**
**Oligonucleotide Microarray Analysis with Single Molecule Sensitivity**
**Jaroslaw Jacak**[1], Jan Hesse[2], Gerhard Regl[3], Thomas Eichberger[3], Fritz Aberger[3], Stefan Howorka[2], Leila Muresan[4], Annemarie Frischauf[3], Gerhard J. Schütz[1].
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We present a microarray analysis platform, which enables detection of hybridized DNA sequences at the level of single molecules. The readout is performed on a high sensitivity chip scanner based on an fluorescence microscope. Capture sequences were printed on custom-made aldehyde-functionalized glass coverslips. The microarray performance was tested with a 60mer fluorescent oligonucleotide hybridized to its complementary sequence, immobilized on the biochip. The determined dynamic range of the platform reaches 4.7 orders of magnitude with a sensitivity of 1.3fM. Furthermore mRNA expression profiling experiments of tetracycline (un)treated HaCat cells were performed. For such competitive hybridization experiments only 5% reverse transcribed cDNA out of 5µg total RNA were hybridized, a hundredfold lower amount then used typically for commercial microarrays. Such wide range in detection sensitivity needs reliable methods for exact data quantification. At low concentration the signal of each spot and molecule brightness was quantified by counting the molecules, fitting them with a 2-dimensional Gaussian function. For high concentrations, the number of molecules per spot was inferred from the total signal per spot. Good correlation of the data with experiments on commercial microarrays using hundredfold higher sample amounts indicates the feasibility of this approach, which avoids application of error prone amplification methods.
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**1600-Pos Board B444**
**Viscosity Measurement of Biological Fluids Using Optical Tweezer**
**Daniel B. Erenso**, Samuel Elrod, Taylor Barns, Anthony Farone, Mary Farone.